Asian Herpetological Research 2019, 10(4): 270–275
DOI: 10.16373/j.cnki.ahr.190028

ORIGINAL ARTICLE

Correlation between Climatic Factors and Genetic Diversity of *Phrynocephalus forsythii*

Yue QI[1], Wei ZHAO[1*], Yongjie HUANG[2], Xiaoning WANG[1] and Yangyang ZHAO[1]

[1] *Gansu Key Laboratory of Biomonitoring and Bioremediation for Environmental Pollution*, *School of Life Sciences*, *Lanzhou University*, *Lanzhou* 730000, *China*
[2] *School of Nature Conservation*, *Beijing Forestry University*, *Beijing* 100083, *China*

Abstract Global climate change is a threat to animals in nearly all biomes and ecosystems, especially for ectotherm whose life activities highly depend on environmental thermal regime. Population genetic diversity which is essential for adaptation to environmental change is a useful index for long-term species survival. In this paper, genetic diversity of eight *Phrynocephalus forsythii* population which distributed in Tarim Basin, China, were evaluated based on three mtDNA gene and its correlation with environment factors were investigated using RDA. Our result revealed that, the level of genetic diversity of *P. forsythii* populations was related to its location but there was no significant correlation between genetic distances and geographic distances in *P. forsythii*. However, we find that mtDNA of *P. forsythii* was subjected to selection pressure during evolution and population genetic diversity was significantly positively related to variation coefficient of rainfall (VCR) and altitude (AL), while significantly negatively related to longitude (N) and annual average temperature (AAT). Our result supported the previous prediction that excessive ambient heat is a threat to *P. forsythii*.

Keywords climatic factors, genetic diversity, selection pressure, *Phrynocephalus forsythii*, Tarim Basin

1. Introduction

Conservation of genetic diversity within species has been recognized as fundamentally important in striving to slow or halt the loss of biodiversity (Xu *et al.*, 2017). The value of genetic diversity is evident from the deleterious impacts of its loss on populations through effects such as increased inbreeding and genetic drift (Oostermeijer *et al.*, 2003). Consequently, the conservation of genetic diversity has become a renewed focus under the expectation that its loss could render populations and species less able to adapt to ongoing environmental change (Georgina, 2003; Mitchell *et al.*, 2015). Genetic diversity is an adaptation parameter of species to environmental changes in the long-term evolutionary process, thus it can be used as an indicator of environmental adaptation: the decrease of genetic diversity can reflect the weaker adaptive ability of organism to potential environmental changes (Frankham *et al.*, 2002). It is generally consider that genetic diversity often affected by numerous factors such as the environmental heterogeneity, habitat suitability and climate fluctuation (Cao *et al.*, 2012; Lv *et al.*, 2014).

Habitat environment plays an important role in the variation of biological genetic diversity (Mitchell *et al.*, 2015). Populations that occupy different environments will be selected by different natural selection. Under the influence of natural selection of the environment, population will retain the best phenotype adapted to the habitat environment, and the genotype frequency associated with the selected phenotype of population will be changed. This process will result in the genetic differences between populations in different natural environments (Ferrari and Chi, 1998). Populations will therefore show extensive geographic variation in genetics along environmental gradients when they occupy different environments (Alkon and Zhao 1993; Chen 1997).

Except for the heterogeneity of environment, natural

*Corresponding author: Dr. Wei ZHAO, from School of Life Sciences, Lanzhou University, China, with his research focusing on evolution and biogeography of reptiles.
E-mail: zhaowei@lzu.edu.cn
Received: 3 May 2019 Accepted: 24 July 2019

selection during extreme events also determine the level of genetic diversity. Phenotypes of individuals are controlled by genotypes and also influenced by environment. So if the genotypes of individuals with different phenotypes differ, genotypes of the individuals which favored by natural selection will tend to increase in relative frequency, and genetic diversity of populations will thus increase. That is to say, natural selection during extreme events brings about genetic changes in population genetics (Jin and Liu, 2008).

Furthermore, stability of climate is also regarded as a selective pressure, and might change the level of genetic diversity (Oliveira *et al.*, 2016). Continual environmental fluctuations provide a background of continuously changing selection pressures to which the species must respond. Such response caused only the individuals whose genotype pre-adapted to the environmental conditions survived during periods of environmental change, hence would influence the level of genetic diversity (Smith *et al.*, 2014; Turchetto-Zolet *et al.*, 2013).

Phrynocephalus forsythii is a viviparous, agamid sand lizard endemic to the Tarim Basin with a broad altitudinal range from 800 to 3000 m (Adler, 1992). A recently study which developed an eco-physiological model of extinction risk under climate change predicted that the *P. forsythii* will face a high risk of extinction due to global warming (Sinervo *et al.*, 2018). In consideration of the crucial role of genetic diversity in future evolutionary trajectory of a specie (Leffler *et al.*, 2012), it is important to understand the variation of population genetic diversity along environmental gradient in *P. forsythii*. Therefore, in this paper, mitochondrial *ND2*, *ND4* and *Cytb* genes were used to evaluate the genetic diversity of *P. forsythii*, and we predicted that the genetic diversity of *P. forsythii* will decrease as the environment temperature increased.

2. Materials and Methods

Ethics statement Animals were treated in accordance with the guidelines of Ethics Committee of the School of Life Sciences, Lanzhou University, that specifically approved this study.

2.1. Population sampling A total of 110 individuals were sampled from eight natural *P. forsythii* populations in Tarim Basin (Figure 1). Animals were euthanized in the field, then dissected and preserved liver and muscle tissue immediately in 100% ethanol. Longitude, latitude and altitude were recorded from the sampling localities and climatic data over 30 years were collected from the nearby weather station (National Climatic Data Center) (Table 1). Variation coefficient of temperature (VCT) was estimated through divided the standard deviation of AAT by the AAT, and variation coefficient of rainfall (VCR) through divided the standard deviation of AAR by the AAR to obtained. Humidity (H) was calculated by AAR/AAT (Kottek *et al.*, 2006).

2.2. DNA extraction and PCR amplification Total genomic DNA was extracted using the TIANamp Genomic DNA Kit. Three loci of mitochondrial genes were amplified using PCR: NADH Dehydrogenase Subunit 2 (*ND2*), NADH Dehydrogenase Subunit 4 (*ND4*) and Cytochrome b (*Cyt-b*). Primers are designed with PRIMER version 6.0. All PCR products were sequenced using the Sanger sequencing method (Genewiz Biotech (Suzhou) Co., Ltd.). All sequences were deposited in the GenBank library (Table S1).

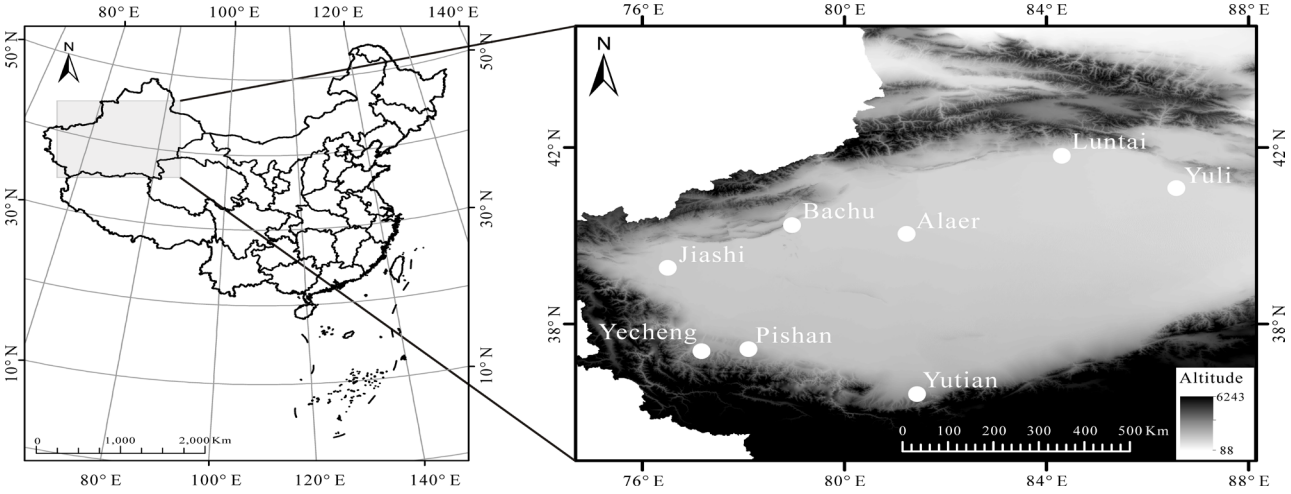

Figure 1 Geographical distribution of sampling points of *P. forsythii*. The elevation gradient is represented by graduated color, from white to black (low to high). The map was downloaded from National Fundamental Geographic Information System (NFGIS)(http://nfgis.gsdi.gov.cn/).

Table 1 Information of P. *forsythii* populations sampled in this study.

Group	Population	Sample size	E	N	AL/m	AAT/°C	VCT/%	AAR/cm	VCR/%	*H*	*h*	π
	Bachu	11	78.96	40.24	1080	11.9	4.2	5.65	71.0	0.475	0.855	0.00173
	Alaer	18	81.23	40.04	1013	10.8	3.7	5.34	41.8	0.494	0.889	0.00228
northern	Jiashi	11	76.50	39.27	1228	12.0	4.2	7.58	66.8	0.632	0.927	0.00819
	Luntai	10	84.30	41.81	963	11.8	4.2	6.82	38.1	0.578	0.844	0.00202
	Yuli	14	86.57	41.08	872	12.0	5.0	5.92	39.4	0.493	0.813	0.00253
	Yecheng	14	77.17	37.38	2007	10.1	6.6	6.66	63.5	0.659	0.978	0.00339
southern	Pishan	16	78.10	37.43	1714	11.1	5.6	5.69	64.5	0.513	0.983	0.00491
	Yutian	16	81.44	36.41	2067	10.2	5.9	5.53	70.2	0.542	0.992	0.01567

Note: Environmental factor abbreviations: E, Latitude. N, Longitude. AL, Altitude. AAT, annual average temperature. AAR, annual average rainfall. VCT, variation coefficient of temperature. VCR, variation coefficient of rainfall. H, humidity.

2.3. Data analysis Sequences were assembled manually using SEQMANII in DNASTAR and aligned with the BioEdit version 7.0.5.2 program (Vrålstad *et al.*, 2002). Nucleotide diversity (π) and haplotype diversity (h) calculation was run in the DnaSP version 5 program(Librado and Rozas, 2009). BI analyses were performed using MrBayes version 3.2.6 (Huelsenbeck & Ronquist 2001) under the AIC with the TrN+I+G model, which was selected by MrModeltest version 3.7 (Posada 1998), for the BI analysis, 20 million generations with Markov Chain Monte Carlo (MCMC) simulations was executed until the average standard deviation of split frequencies dropped below 0.01, and the first 25% were discarded as burn-in. Analysis of molecular variance(AMOVA) was performed to analyze among-population sources of variation using ARLEQUIN version 3.5 (Excoffier *et al.*, 2005) based on the result of Bayesian inference. The genetic distances between populations obtained using a Kimura two-parameter distances (K2P) model of nucleotide substitution available in MEGA version 6.06 (Wang *et al.*, 2007). Statistical analyses of selection was used the CODEML program in PAML version 4.8 (Jin *et al.*, 2018; Yang 2007). We used the site models to detect signatures of selection. Likelihoods were obtained under the following site models: M0 (one ratio), M1A (nearly neutral), M2A (positive selection), M3 (discrete), M7 (beta), M8 (beta & ω). Then Likelihood ratio tests (LRT) were used to compare null model and experimental model: M0-M3, M1A-M2A, M7-M8. Sites were considered to have experienced positive selection when at least one codon model showed $\omega > 1$ and one LRT was significant for either the M1a-M2a or M7-M8 comparisons. The correlation between genetic distance and geographic distance was calculated by Mantel test in R version 3.5.1. Correlation analysis between genetic diversity and environmental factors(Table 1) was conducted in Canoco version 4.5 (Zhang *et al.*, 2012).

3. Result

3.1. Genetic diversity Nucleotide diversity(π) and haplotype diversity (h) ranged from 0.00173–0.01567 and 0.813–0.992, respectively (Table 1). Furthermore, based on the Bayesian analysis, all total of 76 haplotypes from eight populations were divided into two major clades: the southern group (Yutian, Pishan, Yecheng) and the northern group(Jiashi, Alaer, Bachu, Luntai, Yuli), and the genetic diversity of the southern group ($h = 0.995$, $\pi = 0.01496$) was higher than the northern group ($h = 0.974$, $\pi = 0.00858$).

The AMOVA analysis also based on phylogeographic structure. Significance was detected at all three levels, and the among-group component accounted for 71.47% of the total variance (Table 2). Indicate that there is a significant genetic difference between northern and southern group.

3.2. Correlation between the genetic and geographic distances Mantel test revealed that there was no significant correlation between genetic distances and geographic distances ($r = 0.28$, $P = 0.096$). However, genetic distance between populations from northern and southern group was bigger than those from the same group, which indicate that there is a correlation between geographic distance and genetic distance among northern and southern group (Table 3).

3.3. Influence of climatic factors on genetic diversity of *P. forsythii* In the analyses of selection, values of ω much greater than 1 were detected for models M2A, M3 and M8 (Table 4). The LRT that compared M0/M3 was highly significant ($P < 0.001$), which indicated a strong signal of

variable ω among sites. Furthermore, the M1a/M2a LRT and the M7/M8 LRT was significant ($P < 0.05$; Table 4), which indicated that mtDNA of *P. forsythii* was subjected to selection pressure during evolution.

DCA analysis for genetic diversity indicated that the gradient length of the first axis was 0.027, so the RDA model can be selected for sequencing in this study. RDA allows consideration of several environmental factors simultaneously, so it was used for analyzing relationships between environmental factors and genetic diversity. We found strong correlations between genetic diversity of mtDNA and climatic factors, the first axes explained 85.9% of the total variance (Table 5). Nucleotide diversity (π) positively correlated with AL, VCT, VCR and H,

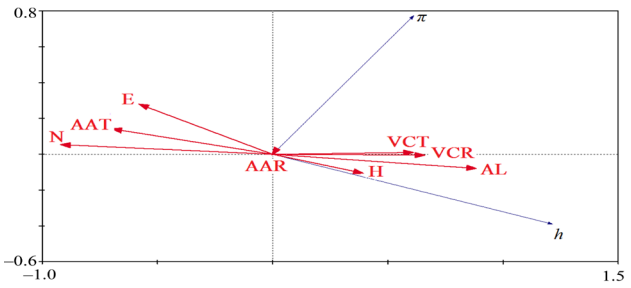

Figure 2 Canonical correspondence analysis of *P. forsythii* and climatic factors. Direction of vectors represent climatic factors accord with genetic diversity, manifest the positive correlation of them; otherwise, they were negative correlation. Length of vectors represents the degree of correlation.

while negatively correlated with E, N, AAT and AAR, and the same correlational relationship was found between haplotype diversity (h) and environmental factors. Furthermore, Monte Carlo Permutation Tests suggested that the correlation of h and π with N ($F = 22.03$, $P = 0.001$), AL ($F = 15.99$, $P = 0.001$), AAT ($F = 5.08$, $P = 0.024$) and VCR ($F = 4.08$, $P = 0.041$) were statistically significant.

Table 2 Analysis of molecular variance (AMOVA) for *P. forsythii* populations.

Source of variation	Percentage of variation	Fixation Indicate
Among groups	71.47	$F_{CT} = 0.71$*
Among populations within groups	17.06	$F_{SC} = 0.60$**
Within populations	11.48	$F_{ST} = 0.89$**

Note: $^{*}P < 0.05$; $^{**}P < 0.01$

Table 3 Mantel test of genetic distance and geographic distance.

Population	Bachu	Yuli	Luntai	Alaer	Jiashi	Yutian	Pishan	Yecheng
Bachu		650.53	460.82	212.28	236.07	477.45	320.75	353.14
Yuli	0.011		202.02	467.48	880.86	681.20	835.00	908.56
Luntai	0.011	0.005		288.36	692.14	597.45	679.83	749.22
Alaer	0.002	0.011	0.011		414.38	408.07	396.97	462.44
Jiashi	0.012	0.014	0.015	0.011		539.28	248.00	219.35
Yutian	0.051	0.050	0.051	0.050	0.051		318.22	395.83
Pishan	0.049	0.048	0.029	0.049	0.049	0.015		82.90
Yecheng	0.050	0.049	0.050	0.049	0.049	0.024	0.018	

Note: genetic distance (below diagonal) and geographic distance (above diagonal)/km

Table 4 Parameters estimation and likelihood ratio tests for the site models.

Model	*ln*L	*k*	Estimates of Parameters	LRT Pairs	*df*	*P*
M0	−8568.323828	11.47455	$\omega = 0.09353$	M0/M3	2	< 0.0001
M3	−8499.367578	11.79313	$p_0 = 0.93890$, $p_1 = 0.06025$, $p_2 = 0.00085$ $\omega_0 = 0.03408$, $\omega_1 = 1.14480$, $\omega_2 = 28.67891$			
M1A	−8502.989659	11.53615	$p_0 = 0.92932$, $p_1 = 0.07068$ $\omega_0 = 0.03057$, $\omega_1 = 1.0000$	M1A/M2A	2	< 0.05
M2A	−8499.551978	11.75374	$p_0 = 0.93014$, $p_1 = 0.06901$, $p_2 = 0.00085$ $\omega_0 = 0.03115$, $\omega_1 = 1.0000$, $\omega_2 = 28.45721$			
M7	−8506.453192	11.58183	$p = 0.06120$, $q = 0.53133$	M7/M8	2	< 0.05
M8	−8502.073392	11.67643	$p_0 = 0.98503$, $p_1 = 0.01497$, $\omega_s = 2.46260$, $p = 0.12035$, $q = 1.44289$			

Note: *ln*L: Log Likelihood, *k*: transition: transversion ratio.

Table 5 Results of ordinations produced by canonical correspondence analysis

Axis	1	2	3	4	Total variance
Eigenvalues	0.859	0.141	0.000	0.000	1.000
Species-environment correlations	1.000	1.000	0.000	0.000	
Cumulative percentage variance of species data	85.9	100.0	0.0	0.0	
Cumulative percentage variance of species-environment relation	85.9	100.0	0.0	0.0	

4. Discussion

Our study examined genetic diversity of *P. forsythii* populations and its relationships with climatic factors at eight sites in Tarim Basin. Although correlation between genetic distances and geographical distances was no statistically significant, genetic diversity of two groups form northern and southern of Tarim Basin existed difference. We considered these differences were caused by selection pressures of different habitat environment, because mtDNA of *P. forsythii* was subjected to selection during evolution and genetic diversity correlated strongly with N and AL through the analysis of RDA. The habitats of *P. forsythii* located in the south of the Tarim basin are characterized by high altitude while the habitats located in the north of the Tarim basin are characterized by low altitude. In the process of adapting to the local ecological environment, population gradually differ in their genome (Ye *et al.*, 2017). Therefore, genetic composition of northern and southern population of *P. forsythii* exist a relatively large variation. Furthermore, the genetic diversity of *P. forsythii* positively correlated with AL while while negatively correlated with N. It indicated that the environmental conditions on the southern side of Tarim basin have lower selection pressure, while the northern side has higher selection pressure for *P. forsythii*. Different selection pressure arising from environmental heterogeneity affects the level of the genetic diversity, and we considered that this selection pressure mainly comes from the higher ambient temperature.

According to the RDA analysis, the level of genetic diversity of *P. forsythii* was negatively correlated with temperature but positively correlated with humidity. According to the cold-climate hypothesis (Qualls and Andrews, 1999), reproductive mode of ectotherms was highly correlated with elevation, with viviparous species occurring at higher elevations than oviparous species, thus viviparity that evolved in *Phrynocephalus* was adaptive to the environment of lower temperature(Wang *et al.*, 2014). In addition, living in localities where ambient temperature is too high, ectotherms trend to retreat to thermal refugia on a daily basis and reduce foraging time, hence, constrains metabolically costly functions such as reproduction, maintenance and growth, and thus affect the level of genetic diversity (Ceia-Hasse *et al.*, 2014). However, the positively correlation between genetic diversity and humidity was not statistic significant in *P. forsythii*.

Finally, it is generally believe that environmental fluctuations is considered as a selection pressure (Hancock *et al.*, 2011; Jin and Liu, 2008). However, our result of RAD run counter to this conclusion, which revealed that the level of genetic diversity of *P. forsythii* positively correlate with variation coefficients of rainfall. Considering that, VCR negatively correlated with AAT, thus the positively correlation between genetic diversity and VCR may be a byproduct of the influence produced by negative correlation between AAT and genetic diversity.

In summary, our research shows that, undergo different nature selection in heterogeneous environment, *P. forsythii* population genetic diversity produced difference, and was mainly affected by N, AL, AAT and VCT. What's more, we find that higher temperature may be a crucial climate factor affecting population genetic diversity of *P. forsythii*.

Acknowledgements The work was supported by the National Natural Science Foundation of China (No. 31471988 and 31200287). We also thank Li Ding from Lanzhou University for the help with data analysis.

Reference

Adle K., Zhao E. M. 1993. Society for the Study of Amphibians and Reptiles. Amphibia-Reptilia, 16: 423–424

Cao M. M., Jin Y. T., Liu N. F., Ji W. H. 2012. Effects of the Qinghai–Tibetan Plateau uplift and environmental changes on phylogeographic structure of the Daurian Partridge (*Perdix dauuricae*) in China. Mol Phylogenet Evol, 65: 823–830

Ceia-Hasse A., Sinervo B., Vicente L., Pereira H. M. 2014. Integrating ecophysiological models into species distribution projections of European reptile range shifts in response to climate change. Ecography, 37: 679–688

Chen Y. J., Wang W., Yang Y. X., Su B., Zhang Y. P., Xiong L. Y., He Z. F., Shu C., Yang D. R. 1997. Genetic Divergence of Cordyceps sinensis as Estimated by Random Amplified Polymorphic DNA Analysis. J Genet Genomics, 24: 410–416

Excoffier L., Laval G., Schneider S. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol Bioinform, 1: 47–50

Frankham R., Ballou J. D., Briscoe D. A. 2002. Introduction to Conservation Genetics: Glossary. Zoologica Africana, 38: 192–192

Ferrari M., Chi M. T. H. 1998. The nature of naive explanations of natural selection. Int J Sci Educ, 20: 1231–1256

Georgina M. 2003. Preserving the tree of life. Science, 300: 1707–1709

Hancock A. M., Witonsky D. B., Alkorta-Aranburu G., Beall C. M., Gebremedhin A., Sukernik R., Sukernik G., Pritchard J. K., Coop G., Rienzo A. D. 2011. Adaptations to Climate-Mediated Selective Pressures in Humans. Plos Genet, 7(4): e1001375

Huelsenbeck J. P., Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, 17:754–755

Kottek M., Grieser J., Beck C., Rudolf B., Rubel F. 2006. World Map of the Köppen-Geiger climate classification updated. Meteorol Z, 15: 259–263

Leffler E. M., Bullaughey K., Matute D. R., Meyer W. K., Ségurel L., Venkat A., Andolfatto P., Przeworski M. 2012. Revisiting an old riddle: what determines genetic diversity levels within species? Plos Biol, 10: e1001388

Librado P., Rozas J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25: 1451–1452

Lv F. H., Agha S., Kantanen J., Colli L., Stucki S., Kijas J.W., Joost S., Li, M. H., Marsan6 P. A. 2014. Adaptations to Climate-Mediated Selective Pressures in Sheep. Mol Biol Evol, 31: 3324–3343

Mitchell M. W., Locatelli S., Clee P. R. S., Thomassen H. A., Gonder M. K. 2015. Environmental variation and rivers govern the structure of chimpanzee genetic diversity in a biodiversity hotspot. BMC Evol Biol, 15: 1–13

Oliveira E. F., Gehara M., Sao-Pedro V. A., Chen X., Myers E. A., Burbrink F. T., Mesquita D. O., Garda A. A., Colli G. R., Rodrigues M. T., Arias F. J., Zaher H., Santos R. M. L., Costa G. C. 2016. Speciation with gene flow in whiptail lizards from a Neotropical xeric biome. Mol Ecol, 24: 5957–5975

Oostermeijer J. G. B., Luijten S. H., den Nijs J. C. M. 2003. Integrating demographic and genetic approaches in plant conservation. Biol Conserv, 113: 389-398

Posada D. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics, 14: 817–818

Qualls C. P., Andrews R. M. 1999. Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor-quality offspring in the lizard, *Sceloporus virgatus*. Biol J Linn Soc, 67: 353–376

Randi E., Alkon P. U. 1994. Genetic Structure of Chukar (*Alectoris chukar*) Populations in Israel. Auk, 111: 416–426

Sinervo B., Miles D. B.,Wu Y. Y., Kirchoff S., Fausto R., Qi Y. 2018. Climate change, thermal niches, extinction risk and maternal-effect rescue of Toad-headed lizards, Phrynocephalus, in thermal extremes of the Arabian Peninsula to the Tibetan Plateau. Integr Zool, 13: 450–470

Smith B. T., McCormack J. E., Cuervo A. M., Hickerson M. J., Aleixo A., Cadena C. D., Pérez-Emán J., Burney C. W., Xie X. O., Harvey M. G., Faircloth B. C., Glenn T. C., Derryberry E. P., Prejean J., Fields S., Brumfield R. T. 2014. The drivers of tropical speciation. Nature, 515: 406–409

Sun R. Y. 2002. Principles of Animal Ecology. Beijing: Beijing Normal University Publishing Group

Turchetto-Zolet A. C., Pinheiro F., Salgueiro F., Palma-Silva C. 2013. Phylogeographical patterns shed light on evolutionary process in South America. Mol Ecol, 22: 1193–1213

Vrålstad T., Myhre E., Schumacher T. 2002. Molecular Diversity and Phylogenetic Affinities of Symbiotic Root-Associated Ascomycetes of the Helotiales in Burnt and Metal Polluted Habitats. New Phytol, 155: 131–148

Wang Y., Zhao L. M., Fang F. J., Liao J. C., Liu N. F. 2013. Intraspecific molecular phylogeny and phylogeography of the *Meriones meridianus* (Rodentia: Cricetidae) complex in northern China reflect the processes of desertification and the Tianshan Mountains uplift. Biol J Linn Soc, 110: 362–383

Wang Z., Lu H. L., Ma L., Ji X. 2014. Viviparity in high-altitude Phrynocephalus lizards is adaptive because embryos cannot fully develop without maternal thermoregulation. Oecologia, 174: 639-649

Xu W. M., Ci X. Q., Li J. 2017. Parallel effects of environmental properties on genetic diversity and species diversity. Biodiversity Science, 25: 481–489

Yang Z. H. 2007. PAML 4: Phylogenetic Analysis by Maximum Likelihood. Mol Biol Evol, 24: 1586–1591

Ye J. W., Zhang Y., Wang X. J. 2017. Phylogeographic history of broad-leaved forest plants in subtropical China. Acta Ecologica Sinica, 37: 5894–5804

Zhang Q., Niu J. M., Wu S. B., Buyantuyev A., Dong J. J. 2012. Impact of climatic factors on genetic diversity of Stipa breviflora populations in Inner Mongolia. Genet Mol Res, 11: 2081–2093

Zhang Q., Xia L., He J. B., Wu Y. H., Fu J. Z., Yang Q. S. 2010. Comparison of phylogeographic structure and population history of two *Phrynocephalus* species in the Tarim Basin and adjacent areas. Mol Phylogenet Evol, 57: 1091–1104

Appendix

Table S1 Sample code and GenBank Accession for mtDNA of *P. forsythii.*

Sampling site	Sample cold	GenBank Accession
Yutian	27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42	MK803651–MK803663, MK803841–MK803856, MK804031–MK804046
Pishan	58, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74	MK803621–MK803636, MK803811–MK803826, MK804001–MK804016
Yecheng	76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89	MK803607–MK803620, MK803797–MK803810, MK803987–MK804000
Bachu	98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108	MK803588–MK803598, MK803778–MK803788, MK803968–MK803978
Luntai	117, 118, 119, 120, 121, 122, 124, 125, 126, 127	MK803571–MK803580, MK803761–MK803770, MK803951–MK803960
Yuli	139, 140, 141, 142, 143, 144, 145, 147, 149, 150, 151, 152, 153, 154	MK803549–MK803562, MK803739–MK803752, MK803929–MK803942
Alaer	175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192	MK803514–MK803531, MK803704–MK803721, MK803894–MK803911
Jiashi	193, 194, 195, 196, 197, 198, 200, 201, 202, 203, 204	MK803503–MK803513, MK803693–MK803703, MK803883–MK803893